REMARKS

Claims 1-124 were pending in the application. Claims 1-29, 36-89 and 93-124were withdrawn from consideration as directed to non-elected inventions. Claims 30, 31, 33 and 34 have been amended. Claims 91 and 92 have been canceled without prejudice.

Claim 30 has been amended to remove dependency from claim 1 and to add a specific level of homology. Claim 31 has been amended to remove Markush language. Claims 33 and 34 have been amended to remove Markush language and to further clarify the claimed polypeptide. Claim 90 has been amended to add a specific level of homology. Support for the claim amendments can be found throughout the specification as filed.

Claims 91 and 92 have been canceled without prejudice to presentation in related applications. Claims 91 and 92 contained subject matter substantially identical to subject matter recited in claims 30, 31, 33 and 34, as amended.

The specification has been amended to correct line spacing and font size in Tables 1-5.

Upon entry of this amendment, claims 30, 32, 33, 35 and 90 will be pending.

No new matter has been added.

Specification

The Examiner noted that Tables 1-5 in the specification failed to comply with 37 CFR § 1.52(b) in that the line spacing was not 1.5 or 2.0. Further, the Examiner noted that the typewritten characters should be in a font "having capital letters at least 0.21 cm (0.08 inch) high. As required by the Examiner, Applicants have amended Tables 1-5 to correct line spacing (from 1.0 spacing to 1.5 spacing) and font size (from a font having capital letters 0.2 cm to a font having capital letters at least 0.21 cm in height).

In view of the foregoing, Applicants respectfully request that the objections to the specification be withdrawn.

Objections

Claims 30, 31 and 33-35 stand objected to as allegedly reciting an improper Markush group. Claims 30, 31, 33 and 34 have been amended to remove Markush language and to specifically claim the elected species, thereby rendering the objection moot. Claim 35 depends from claim 34. Applicants reserve the right to re-enter claims directed at non-elected species.

Claims 30, 31, 33-35 and 90-92 stand objected to as allegedly failing to further limit the subject matter of a previous claim. Claims 30, 31, 33, 34 and 90 have been amended to limit the subject matter of a previous claim. Claim 35 depends from claim 34.

Claim 91 stands objected to as allegedly being a substantial duplicate of claim 90. Claim 91 has been canceled without prejudice.

In view of the foregoing, Applicants respectfully request that the objections be withdrawn.

Rejection under 35 U.S.C. § 101

Claims 30, 31, 33-35 and 90-92 stand rejected under 35 U.S.C. § 101 because the claimed invention is allegedly "drawn to an invention with no apparent or disclosed specific and substantial credible utility . . . The instant application does not disclose a specific biological role for this protein or its significance to a particular disease, disorder of [sic] physiological process which one would wish to manipulate for a desired clinical effect" (Office Action, page 5). Further, the Office alleges that "because the instant specification has failed to credibly identify a physiological process which has been shown to be influenced by the activation or inhibition of a putative receptor protein of the instant invention an artisan would have no way of predicting what effects the administration of that ligand to an organism would have." (Office Action, page 6). Applicants respectfully disagree.

Preliminarily, Applicants note that claims 30, 31, 33, 34 and 90 have been amended. Claims 91 and 92 have been canceled without prejudice.

Utility Examination Guidelines

The Utility Examination Guidelines require a claimed invention to have a utility that is specific to the subject matter claimed (a "specific utility"). The present application recites at, for example, paragraph [000124] that "G proteins may be isolated using . . . isolated G protein-coupled receptors. Similarly, G proteins may be detected in a sample isolated using . . . isolated G protein-coupled receptors." The specification also teaches that the claimed invention can be used, *inter alia*, to identify ligands and/or protein binding partners. Additionally, the polypeptides of the present invention can be used to generate antibodies useful to localize the protein *in vivo* or *in vitro*. Being able to identify specific cell types is also useful for identifying defects and abnormalities in the tissues by the absence or presence of staining the nGPCR. Thus, there is no question that Applicants have asserted at least one specific utility and, in fact, have provided numerous specific utilities for the polypeptides of the present invention.

Additionally, the Office appears to be under the assumption that ***absolute*** certainty is required for a polynucleotide to have a specific utility. The standard applicable in this case is not, however, proof to certainty, but rather proof to reasonable probability. As the Supreme Court stated, applicant need only prove a "substantial likelihood" of utility; certainty is not required. *Brenner v. Manson*, 383 U.S. at 532. Although there may be numerous inventions that may arise from the present application, this standard does not justify the Office's stance that the present invention lacks a specific utility. Thus, Applicants have complied with the specific utility requirement.

The Claimed Invention Has A Substantial Utility

The Utility Examination Guidelines also require that a claimed invention have a utility that defines a real-world use (a "substantial utility"). Applicants teach, as described above, that the claimed invention can be used to make antibodies, identify ligands and other binding partners, such as other proteins that interact with the polypeptide (*i.e.*, a G protein). Thus, it is clear that the claimed invention has real-world uses. All the uses

described in the present application are real-world uses and stand in stark contrast to the "throw away" uses (*e.g.*, landfill component or snake food) set forth in the utility guidelines.

Applicants further note that GPCRs, ORF clones of GPCRs, and antibodies that bind to GPCRs are commercially available. For example, Applicants point out that FabGennix Inc. of Shreveport, Louisiana sells an antibody directed to Retinal Anti-GP75. GPCR75 is said to be a GPCR for which a ligand has not yet been identified (*see* attached product sheet). Invitrogen sells ORF clones of GPCRs including those for which a ligand has not yet been identified (*see* attached list, especially noting Clone Ids IOH22483, IOH14039, IOH13056, IOH22637, IOH13239, and IOH13516). MD Bio of Taiwan sells GPCR peptides and antibodies against such peptides, again where no ligand has yet been identified. That at least three companies make and sell such GPCR products proves that there is a well-established utility for the presently claimed GPCR polypeptides.

Thus, there is no question that Applicants have asserted at least one substantial utility and, in fact, have provided numerous substantial utilities. Accordingly, Applicants have complied with the "substantial utility" requirement.

The Claimed Invention Has A Credible Utility

In addition to a specific and substantial utility, the Utility Examination Guidelines require that such utility be credible (a "credible utility"). That is, whether the assertion of utility is believable to a person of ordinary skill in the art based on the totality of evidence and reasoning provided. Clearly, the numerous specific and substantial utilities asserted by Applicants are credible.

Assertions of utility are credible unless "(A) the logic underlying the assertion is seriously flawed, or (B) the facts upon which the assertion is based is inconsistent with the logic underlying the assertion." (See, Revised Interim Utility Guidelines Training Materials.) Further, the PTO is reminded that it ***must*** treat as true a statement of fact made by Applicants in relation to an asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis

to doubt the credibility of such a statement. All the utilities described for the polypeptide are based on sound logic. Furthermore, the utilities for the claimed polypeptides are ***not*** inconsistent with the logic underlying the assertion that the polypeptides are useful. Polypeptides are useful to generate antibodies, identify ligands or protein partners, evaluate expression patterns, evaluate protein activity, *etc.* The Office has provided no evidence that the logic is seriously flawed or that the facts upon which these assertions are based are inconsistent with the logic underlying the assertions.

Furthermore, GPCR proteins have a well-established utility. Many medically significant biological processes are mediated by signal transduction pathways involving G-proteins and other second messengers, and G protein coupled seven transmembrane receptor proteins are recognized as important therapeutic targets for a wide range of diseases. According to a recently issued United States patent, nearly 350 therapeutic agents targeting GPCRs have been successfully introduced onto the market in only the last fifteen years. (*See*, U.S. Patent No. 6,114,127, at col. 2, lines 45-50.) A recent journal review reported that most GPCR ligands are small and can be mimicked or blocked with synthetic analogues. That, together with the knowledge that numerous GPCRs are targets of important drugs in use today, make identification of GPCRs "a task of prime importance." (*See*, Marchese et al., Trends Pharmacol. Sci., 20(9): 370-5, 1999, attached hereto). Thus, the allegation that there is no well-established utility for proteins of the class that the Applicants are now claiming is directly refuted by industry evidence.

In this respect, the G protein coupled receptor family is analogous to the chemical genus that was the subject of *In re Folkers*, 145 USPQ 390 (CCPA 1965) (Compound that belongs to class of compounds, members of which are recognized as useful, is considered useful under §101.) The Patent Office does not serve the public by attempting to substitute a formulaic analysis of § 101 for the established judgment of the biopharmaceutical industry as to what is "useful." If the Patent Office is aware of any well-grounded scientific literature suggesting that GPCRs are not useful, Applicants request that it be made of record.

Art-Recognized Utility

The Utility requirement may also be satisfied by an "Art Established Utility" which means that "a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention... and the utility is specific, substantial and credible." (M.P.E.P. §2107).

Applicants note for the record that the Patent Office apparently agrees with Applicants' reasoning that GPCRs are useful in that the Office has granted and apparently continues to grant patents to G-protein coupled receptors, their encoding polynucleotides and antibodies directed to them ***in which no natural substrate or specific biological significance*** is ascribed to the GPCR. Specifically, Applicants would like to bring the following US Patents to the Office's attention:

6,518,414 Maclennan "Molecular Cloning and Expression of G-Protein Coupled Receptors" (Claims an isolated polynucleotide)
6,511,826 Li et al. "Polynucleotides Encoding Human G-Protein Chemokine Receptor (CCR5) HDGNR10" (Claims an isolated polynucleotide encoding a protein identified as a "chemokine receptor" with no specific chemokine identified)
6,372,891 Soppet et al. "Human G-Protein Receptor HPRAJ70" (Claims an antibody directed to a G-protein coupled receptor)
6,361,967 Agarwal et al. "AXOR10, A G-Protein Coupled Receptor" (Claims an isolated polynucleotide)
6,348,574 Godiska et al. "Seven Transmembrane Receptors" (Claims an antibody directed to a G-protein coupled receptor)
6,114,139 Hinuma et al. "G-Protein Coupled Receptor Protein and A DNA Encoding the Receptor" (Claims an isolated polynucleotide).
6,111,076 Fukusumi et al. "Human G-Protein Coupled Receptor (HIBCD07)" (Claims isolated polypeptide)
6,107,475 Godiska et al. "Seven Transmembrane Receptors" (Claims isolated polynucleotide and methods)
6,096,868 Halsey et al. "ECR 673: A 7-Transmembrane G-Protein Coupled Receptor" (Claims isolated polypeptide)
6,090,575 Li et al. "Polynucleotides Encoding Human G-Protein Coupled Receptor GPR1" (Claims isolated polynucleotide)
6,071,722 Elshourbagy et al. "Nucleic Acids Encoding A G-Protein Coupled 7TM Receptor (AXOR-1)" (Claims an isolated polynucleotide)
6,071,719 Halsey et al. "DNA Encoding ECR 673: A 7-Transmembrane G-Protein Coupled Receptor" (Claims an isolated polynucleotide)
6,060,272 Li et al. "Human G-Protein Coupled Receptors" (Claims isolated polynucleotide)

6,048,711 Hinuma et al. "Human G-Protein Coupled Receptor Polynucleotides" (Claims isolated polynucleotide)
6,030,804 Soppet et al. "Polynucleotides Encoding G-Protein Parathyroid Hormone Receptor HLTDG74 Polypeptides" (Claims isolated polynucleotide)
6,025,154 Li et al. "Polynucleotides Encoding Human G-Protein Chemokine Receptor HDGNR10" (Claims an isolated polynucleotide encoding a protein identified as a "chemokine receptor" with no specific chemokine identified)
5,998,164 Li et al. "Polynucleotides Encoding Human G-Protein Coupled Receptor GPRZ" (Claims isolated polynucleotide)
5,994,097 Lal et al. "Polynucleotide Encoding Human G-Protein Coupled Receptor" (Claims isolated polynucleotide)
5,958,729 Soppet et al. "Human G-Protein Receptor HCEGH45" (Claims isolated polypeptide)
5,955,309 Ellis et al. "Polynucleotide Encoding G-Protein Coupled Receptor (H7TBA62)" (Claims isolated polynucleotide)
5,948,890 Soppet et al. "Human G-Protein Receptor HPRAJ70" (Claims isolated polypeptide)
5,945,307 Glucksmann et al. "Isolated Nucleic Acid Molecules Encoding A G-Protein Coupled Receptor Showing Homology to The 5HT Family of Receptors" (Claims isolated polynucleotide)
5,942,414 Li et al. Polynucleotides Encoding Human G-Protein Coupled Receptor HIBEF51" (Claims isolated polynucleotide)
5,912,335 Bergsma et al. "G-Protein Coupled Receptor HUVCT36" (Claims isolated polynucleotide)
5,874,245 Fukusumi et al. "Human G-Protein Coupled Receptors (HIBCD07)" (Claims isolated polynucleotide)
5,871,967 Shabon et al. "Cloning of A Novel G-Protein Coupled 7TM Receptor" (Claims isolated polynucleotide)
5,869,632 Soppet et al. "Human G-Protein Receptor HCEGH45" (Claims isolated polynucleotide)
5,856,443 MacLennan et al. "Molecular Cloning and Expression of G-Protein Coupled Receptors" (Claims isolated polynucleotide)
5,834,587 Chan et al. "G-Protein Coupled Receptor, HLTEX11" (Claims isolated polypeptide)
5,776,729 Soppet et al. "Human G-Protein Receptor HGBER32" (Claims isolated polynucleotide)
5,763,218 Fujii et al. "Nucleic Acid Encoding Novel Human G-Protein Coupled Receptors" (Claims isolated polynucleotide)
5,756, 309 Soppet et al. "Nucleic Acid Encoding A Human G-Protein Receptor HPRAJ70 and Method of Producing the Receptor" (Claims isolated polynucleotide)
5,585,476 MacLennan "Molecular Cloning and Expression of G-Protein Coupled Receptors" (Claims isolated polynucleotide)
5,759,804 Godiska et al. "Isolated Nucleic Acid Encoding Seven Transmembrane Receptors" (Claims isolated polynucleotide and methods)

Applicants submit that these issued US Patents are evidence of an art recognized utility for G-protein coupled receptors whose natural ligand is unknown. If the Patent Office's position is that issued patents are ***not*** sufficient evidence of art recognition then Applicants respectfully request that this position be made of record. In the alternative, if the Patent Office wishes to take the position that these issued patents are directed to non-statutory subject matter, then Applicants respectfully request that this position be made of record as well.

Applicants respectfully assert that identification of a "physiological process which has been shown to be influenced by the activation or inhibition of a putative receptor protein of the instant invention" is not necessary for showing the utility of the present invention. As discussed above, clones of GPCRs, GPCRs, and antibodies to GPCRs (where a ligand or a physiological process linked thereto has yet to be identified) are commercially available. The commercial availability of such products is evidence that those skilled in the art would immediately appreciate that the invention is useful based on the characteristics of the invention and that the utility is specific, substantial and credible.

In view of the foregoing, Applicants respectfully requests that the rejection under 35 U.S.C. § 101 be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 30, 31, 33-35 and 90-92 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to adequately teach how to use the instant invention. According to the Office, "Since the claimed invention is not supported by a specific, substantial, and credible asserted utility or a well established utility...one skilled in the art clearly would not know how to used the claimed invention." (Office Action, page 8) Applicants respectfully disagree.

As discussed above, the present invention ***is*** supported by a specific, substantial, and credible asserted utility as well as a well-established utility. Accordingly, Applicants respectfully request that the rejection be withdrawn.

Claims 30-35 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly subject matter which was not was described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, has possession of the claimed invention. According to the Office:

> These claims encompass an isolated polypeptide comprising an amino acid sequence 'homologous' to the amino acid sequence presented in SEQ ID NO:67 of the instant application. The instant specification, however, only contains an adequate written description of a single protein within the recited genus and this protein comprises the amino acid sequence presented in SEQ ID NO:67. No homologous protein is described in the instant specification...Whereas the instant claims encompass a potentially large genus of isolated polypeptides comprising different amino acid sequences, the instant specification does not provide a detailed description of a sufficient number of species of nucleic acids with[in] the claimed genus to establish possession of that genus, The description of a single isolated nucleic acid encoding a single protein does not serve as an adequate basis for the relatively large genus of nucleic acids encompassed by the instant claims.

(Office Action, page 8). Applicants respectfully disagree.

According to the M.P.E.P:

> An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention.

(M.P.E.P § 2163). The pending claims demonstrate that Applicants were in possession of the claimed invention at the time the application was filed because the claims contain "descriptive means" that define the claimed invention.

For example, claim 30, as amended, recites an isolated polypeptide comprising an amino acid sequence with at least 80% sequence identity to a sequence of SEQ ID NO:67, or a fragment thereof comprising at least 10 consecutive amino acids of SEQ ID NO: 67.

Claim 33, as amended, recites "The polypeptide of claim 30 wherein said polypeptide comprises an epitope specific to SEQ ID NO:67." The term "epitope specific" is defined on page 23 of the present application and would be readily

understood by one of ordinary skill in the art to provide structural and functional characteristics of the invention. Claim 30, as amended, refers to isolated polypeptides that are at least 80% identical to SEQ ID NO:67. Claim 90, as amended, refers to an isolated polypeptide that is at least 95% identical to SEQ ID NO:67 an comprises an epitope specific to nGPCR-93. Claim 31, as amended, refers to a peptide that is identical to SEQ ID NO:67. Claim 34, as amended, recites a polypeptide of claim 30 wherein said polypeptide comprises at least one extracellular domain of nGPCR-93. Claim 35 is dependent on claim 34.

A person of ordinary skill in the art would understand that the "descriptive means" of the pending claims include that the isolated polypeptide is at least 80% identical to or at least 95% identical to SEQ ID NO:67. A person of ordinary skill in the art would understand that further "descriptive means" of the genus include the epitope specificity and the presence of an extracellular domain of the polypeptide.

The "descriptive means" set forth in pending claims allow a person of ordinary skill in the art to readily understand that the defining characteristics of the claimed invention. A person of ordinary skill in the art would understand that Applicants had possession of the claimed invention. The "descriptive means" set forth clearly show possession of the claimed invention.

Notwithstanding the foregoing, Applicants respectfully assert that Example 14 of the Revised Interim Written Description Guidelines Training Materials indicates that a disclosure of a single species, a 95% level of homology, and a functional limitation is adequate to satisfy the written description requirement for a claimed genus. Claims 33 and 90 each provide a single species within the genus, at least a 95% level of homology, and a functional limitation.

Thus, Applicants have clearly shown that they had possession at the time the application was filed. Accordingly, Applicants respectfully request that the rejections under 35 U.S.C. § 112, first paragraph be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

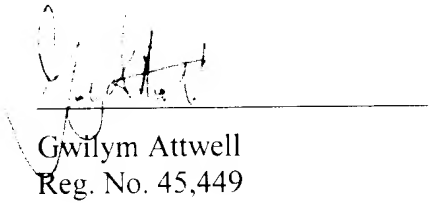
Claims 30, 31, 33-35 and 90-92 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. According to the Office, the claims are indefinite in so far as they rely upon the terms "homologous" and "nGPCR-x" (Office Action, page 10). Applicants respectfully disagree.

Although Applicants respectfully assert that a skilled artisan would readily understand the instant usage of the terms "homologous" and "nGPCR-x", in order to advance prosecution Applicants have amended the claims to further clarify the claim language, thereby rendering the rejection moot. In view of the foregoing, Applicants respectfully request that the rejection under 35 U.S.C. § 112, second paragraph, be withdrawn.

Conclusion

Applicants believe the claims are in condition for allowance. An early Notice of Allowance is therefore earnestly solicited. Applicants invite the Examiner to contact the undersigned at (215) 665-6904 to clarify any unresolved issues raised by this response.

Respectfully submitted,



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Attachments: Marchese et al., Trends Pharmacol. Sci., 20(9):370-5, 1999
Product Sheet for Anti-GPCR-75 Antibodies
Product sheet for GPCR control peptides and antibodies (MD Bio)
Product sheet for GPCR ORF clones (Invitrogen)

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Novel GPCRs and their endogenous ligands: expanding the boundaries of physiology and pharmacology

Adriano Marchese, Susan R. George, Lee F. Kolakowski Jr, Kevin R. Lynch and Brian F. O'Dowd

Nearly all molecules known to signal cells via G proteins have been assigned a cloned G-protein-coupled-receptor (GPCR) gene. This has been the result of a decade-long genetic search that has also identified some receptors for which ligands are unknown; these receptors are described as orphans (oGPCRs). More than 80 of these novel receptor systems have been identified and the emphasis has shifted to searching for novel signalling molecules. Thus, multiple neurotransmitter systems have eluded pharmacological detection by conventional means and the tremendous physiological implications and potential for these novel systems as targets for drug discovery remains unexploited. The discovery of all the GPCR genes in the genome and the identification of the unsolved receptor–transmitter systems, by determining the endogenous ligands, represents one of the most important tasks in modern pharmacology.

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The G-protein-coupled receptors (GPCRs) are transducers of extracellular messages and they allow tissues to respond to a wide array of signalling molecules. Most of the endogenous ligands are small and the binding of these ligands to their receptor(s) can be mimicked (or blocked) by synthetic analogues. Together with the knowledge that numerous GPCRs are targets of important drugs in use today, GPCR identification is a task of prime importance. In the 14 years since the first cloning of genes for GPCRs, most of the molecules known to signal cells via the heterotrimeric G-protein–effector systems have been assigned a cloned GPCR gene. However, the vigorous search for novel GPCR genes has far outpaced the identification of novel endogenous ligands. A group of genes has been identified whose products are, using the criterion of sequence similarity, members of the GPCR family but for which the ligands are not known, and these are commonly known as orphans (oGPCR).

The GPCR gene family is the largest known receptor family (see Box 1) and shares a common secondary structure that consists of seven transmembrane domains. Setting aside the odorant receptors (encoded by hundreds of genes), nearly 300 mammalian GPCR genes have been recognized[1]. On the basis of structure, the GPCRs can be separated into three subfamilies. The inclusion of a receptor in a subfamily requires the presence of an overall percentage amino acid identity and not any discrete motif. Most GPCRs, including the odorant receptors, are grouped in Family A. Several additional GPCRs, which have as their ligands peptides such as secretin, vasoactive intestinal peptide and calcitonin, make up Family B. Family C comprises the metabotropic glutamate receptors, the Ca^{2+}-sensing receptor, pheromone receptors, the $GABA_B$ receptors and the taste receptors. Within each family, GPCRs are grouped by sequence similarity and ligand specificity; approximately one third of Family A members

Box 1. How big is the GPCR family?

The size of the GPCR family surprised even the most optimistic pharmacologist as many subfamilies proved to be larger than had been predicted by classical pharmacological techniques. Furthermore, some ligands that were not widely considered to signal via receptors (e.g. nucleotides) are recognized now to have numerous receptor subtypes. The discovery of these multiple subtypes, new ligands and the rapid accumulation of novel GPCR sequences have led to the expectation that many more mammalian GPCRs await discovery. Thus, an obvious question to ask is: how many GPCR genes are there in the human genome? Although simply waiting a few years should answer this question directly, there are practical implications in making an educated guess now. For example, is the receptor for a candidate ligand likely to be visible now among the existing oGPCR DNAs? And, is further searching for oGPCR DNAs a worthwhile endeavour?

The recent completion of the nematode (*Caenorhabditis elegans*) translated genome provides an interesting comparison to mammalian GPCRs. In contrast to the single cell yeast (with its two GPCR genes), multicellularity obviously demands cell-to-cell communication and the added complexity imposes a requirement for a much larger repertoire of GPCRs. According to the analysis reported by Bargmann[1], 5% of the 19 100 nematode genes encode GPCRs. Their distribution among GPCR families is reminiscent of the mammalian GPCR genes, some 700–1000 chemoattractant (odorant) genes (including numerous pseudogenes), approximately 150 Family A genes and four-to-five each Family B and C genes. By analogy, this suggests that the number of mammalian GPCRs could total 5000 (5% of mammalian genes estimated to be 80 000–100 000). Unfortunately, the *C. elegans* genome provides no direct clues for oGPCR identification as the closest nematode GPCR is <35% identical to any mammalian GPCR and there are no obvious homologues to mammalian pre-pro-neuropeptide genes. In contrast, the accumulation of nucleotide sequence information from another surrogate organism, the zebrafish (*Danio rerio*), should be more informative because the conceptualized GPCR amino acid sequences are often ~70% identical to orthologous mammalian GPCRs.

Reference

1 Bargmann, C. (1998) *Science* 282, 2028–2033

are oGPCRs and this review will focus on these receptors. Thus, in a decade, the list of signalling molecules for which the GPCR genes had not been cloned has been supplanted by a list of ~80 oGPCRs awaiting a ligand (see Table 1). The characterization of these GPCRs has already enabled the discovery of several new endogenous ligands; this will be discussed later.

Novel GPCR gene discovery

Very few GPCRs have been purified, thus the pace of GPCR gene discovery has been fuelled by a series of highly successful cloning techniques. The identification (using amino acid sequence determination and expression cloning) of a few sequences encoding Family A GPCRs demonstrated that these were related genes[1]. Cloning by low stringency hybridization to cDNA/genomic DNA libraries yielded a stream of novel GPCR DNAs. The pace of discovery quickened with the use of the polymerase chain reaction (PCR). The database of expressed sequence tagged cDNAs (ESTs) has provided material for a further expansion of Family A, as has the high-throughput sequencing of 100–200 kb pair segments of human DNA.

Novel GPCR identification

Many oGPCRs are found to be similar to known GPCRs. Where the identity reaches the threshold of ~45%, it is likely that the receptors will share a common ligand, i.e. that the oGPCR will be a pharmacological subtype of the known GPCR. This rule is not without exception. Take, for example the orphanin FQ/nociceptin receptor; this has ~65% amino acid identity to opioid receptors, but does not have high affinity for opioid peptides[2,3]. Many GPCR subtypes have <40% amino acid identity, in which case sequence comparison might not be profitable. Moreover, because the ligand-binding pocket has not yet been described fully for any receptor, it is not feasible to predict ligand identity. However, dendritic tree building shows that receptors that respond to the same, or similar, agonists often cluster. For example, most members of the prostanoid receptor subfamily share <30% amino acid identity, yet these eight receptors are more like one another than any other GPCR. A similar situation exists among the nucleotide receptors, chemokine receptors and other cationic amine receptors. In the way that many known GPCRs fall into subfamilies, many oGPCRs cluster together, sometimes with members having >50% amino acid identity, which suggests that the problem of the ~80 oGPCRs might be solved by a mere 30 or 40 ligands. For example, the recent identification of Edg-1 as a sphingosine 1-phosphate receptor[4–6] leads directly to the prediction that Edg-3 and Edg-5 (both >50% identical to Edg-1) have the same ligand. More distant members of the Edg cluster, Edg-2 and Edg-4 are known to be receptors for the structurally related ligand, lysophosphatidic acid[7–9].

When homology does not inform, i.e. the nearest known GPCR has <35% amino acid identity to the orphan, ligand identification is challenging. There are no signature amino acids that predict either the nature of the ligand or the identity of the interacting Gα subunit type(s). In those cases where the ligand is a molecule with an established pharmacology, tissue distribution has allowed inference of ligand identity. Thus, an important clue to identifying the oGPCR RDC-8 as encoding the adenosine A_{2A} receptor was the concordance of *in situ* hybridization and ligand ([^{3}H]CGS21680) autoradiography signals in rat brain sections[10]. Similarly, the occurrence of both cannabinoid binding sites and SKR6 receptor mRNA accumulation in NG108 cells led to the identification of the cannabinoid CB_1 receptor[11].

Table 1. Amino acid sequence identity of some orphan G-protein-coupled receptors

Homology	Name	Species	% Amino acid identity	Accession no.
Opioid and somatostatin receptor-like	GPR7	Human	62% GPR8, 40% sst_4	U22491
	GPR8	Human	62% GPR7, 45% sst_4	U22492
	GPR24	Human	33% sst_5, 32% sst_4	U71092
	GPR14	Rat	29% μ-opioid, 28% sst_4	U32673
	GPR54	Rat	37% gal2, 35% GAL1	AF115516
Chemokine receptor-like	GPR2	Human	41% CXCR3, 40% CCR7	U13667
	CKRX	Human	53% E01, 43% CCR1	AF014958
	E01	Mouse	53% CKRX, 36% CCR1	AF030185
	MIP-1αRL1	Mouse	62% CCR1, 50% CCR3	U28405
	GPR28	Human	43% CCR7, 38% CCR6	U45982
	STRL33	Human	37% CCR7, 37% CCR8	U73529
	PPR1	Bovine	39% CCR7, 37% GPR28	S63848
	g10d	Rat	33% RDC1, 30% CCR9	L09249
	RDC1	Human	33% g10d, 30% CXCR2	X14048
	TM7SF1	Human	22% GPR5, 14% CCR6	AF027826
	CLR1	Chicken	51% BLR1, 36% CXCR1	AF029369
Chemoattractant receptor-like	Dez	Human	37% GPR1, 35% FPR2	U79527
	FPRL2	Human	72% FPR2, 56% FPR1	M76673
	FPR2	Human	72% FPRL2, 69% FPR1	M76672
	GPR1	Human	37% Dez, 34% FPR2	U13666
	GPR30	Human	32% FPRL2, 32% FPR2	AF027956
	GPR32	Human	39% FPR1, 35% FPRL2	AF045764
	GPR33	Mouse	36% GPR32, 36% Dez	AF045766
	GPR44	Human	37% Dez, 36% FPRL2	AF118265
	mas oncogene	Human	34% MRG, 26% C5aR	M13150
	MRG	Human	34% *mas* oncogene, 34% C5aR	S78653
	RTA	Rat	32% *mas* oncogene, 33% MRG	M32098
	GPR53p	Human	35% MRG, 28% *mas* oncogene	AF096785
Angiotensin receptor-like	GPR15	Human	34% GPR25, 31% APJ	U34806
	GPR25	Human	34% GPR15, 32% APJ	U91939
Cannabinoid receptor-like	GPR3	Human	59% GPR6, 57% GPR12	U13668
	GPR6	Human	59% GPR3, 56% GPR12	L36150
	GPR12	Rat	57% GPR3, 56% GPR6	U18548
	EDG-6	Human	46% EDG-3, 44% EDG-1	AJ000479
GPR4 receptor-like	OGR1	Human	48% GPR4, 35% TDAG8	U48405
	GPR4	Human	48% GPR12A, 36% TDAG8	L36148
	TDAG8	Human	36% GPR4, 35% GPR12A	U95218
	G2A	Mouse	34% GPR4, 31% OGR1	AF083442
Neuropeptide Y receptor-like	GIR	Mouse	35% GPR10, 30% NK_4	M80481
	GPR19	Human	27% GAL1, 26% NPY Y_2	U64871
	GPR22	Human	26% NPY Y_5, 24% CCK$_1$	U66581
Amine receptor-like	PNR	Human	33% 5-HT_4, 33% 5-HT_7	AF021818
	GPR26	Human	28% 5-HT_{5B}, 23% 5-HT_{5A}	
	GPR27	Mouse	29% D4, 25% 5-HT_6	AF027955
	AGR9	Rat	24% H_2, 24% NK_2	S73608
	GPR21	Human	27% β_3AR, 24% β_2AR	U66580
	PSP24	Human	26% 5-HT_4, 23% β_1AR	U92642
	GPR45	Human	70% PSP24, 21% NK_2	AF118266
	A-2	Human	21% 5-HT_{1F}, 19% 5-HT_{1E}	U47928
	GPR52	Human	71% GPR21, 27% H_2	AF096784
	RE2	Human	25% α_{1A}AR, 25% α_{1C}AR	AF091890
	GPR57	Human	59% GPR58, 37% PNR	N/A
	GPR58	Human	59% GPR57, 42% PNR	N/A
	GPR61	Human	27% LZY2, 30% 5-HT_6	N/A
	GPR62	Human	27% LZY, 28% 5-HT_6	N/A
P2 receptor-like	GPR23	Human	53% RBintron, 33% $P2Y_{10}$	U66578
	RBintron	Human	53% GPR23, 38% $P2Y_4$	L11910
	GPR35	Human	32% GPR23, 30% HM74	AF027957
	$P2Y_{10}$	Human	34% RBintron, 33% GPR23	AF000545
	GPR17	Human	35% $P2Y_2$, 34% $P2Y_4$	U33447
	GPR18	Human	30% RBintron, 29% GPR17	L42324
	HM74	Human	36% GPR31, 29% $P2Y_1$	D10923
	GPR31	Human	36% HM74, 29% $P2Y_1$	U65402

Table 1. (*cont.*)

Homology	Name	Species	% Amino acid identity	Accession no.
P2 receptor-like (*cont.*)	RSC338	Human	33% H963, 28% tp2γ	D13626
	EBI 2	Human	33% RBintron, 30% CCR1	L08177
	H963	Human	33% RSC338, 28% PAFR	AF002986
	GPR41	Human	98% GPR42, 41% GPR43	AF024688
	GPR42	Human	98% GPR41, 28% GPR23	AF024689
	GPR40	Human	31% GPR43, 26% CXCR1	AF024687
	GPR43	Human	41% GPR41, 31% GPR40	AF024690
	GPR20	Human	31% $P2Y_4$, 26% GPR23	U66579
	GPR34	Human	31% RSC338, 29% RBintron	AF118670
	GPR55	Human	29% $P2Y_5$, 30% GPR23	AF096786
Neurotensin receptor-like	GHS-R	Human	35% NTS1, 33% nts2	U60179
	GPR39	Human	32% NTS1, 25% nts2	AF034633
	HSOGPCR2	Human	38% GPR38, 34% GHS-R	AF044601
Melatonin receptor-like	H9	Human	48% ML_{1A}, 45% ML_{1B}	U52219
Endothelin receptor-like	GPR37	Human	68% ET_BR-LP-2, 27% ET_B	U87460
	ETBR-LP-2	Human	68% GPR37, 27% ET_B	Y16280
Glycoprotein hormone receptor-like	LGR5	Human	26% FSH-R, 25% LH-R	AF062006
Opsin receptor-like	Encephalopsin	Human	32% Peropsin, 31% Rhodopsin	AF140242
	RGR	Human	27% Peropsin, 26% Rhodopsin	U15790

Please refer to the *TiPS Receptor and Ion Channel Nomenclature Supplement* and to individual GenBank accession numbers for further information.

Endogenous ligand identification

In the same way that EST database searching has yielded GPCR DNAs, it has also yielded DNAs encoding peptide sequences related to known peptides. Several novel chemokines have been discovered using this approach and these have proven to be the ligands for several chemokine receptors. For example, a CC chemokine termed ELC (EBI-ligand chemokine) was identified from the EST database and found to be the endogenous ligand for the orphan receptor EBI1, which has since been renamed CCR7 (Ref. 12). Similarly, the CC chemokine liver and activation-regulated chemokine (LARC) was identified from the EST database[13] and subsequently shown to be the ligand for the orphan STRL22 receptor; this was renamed CCR6 (Refs 14–16). Another EST encoding a CXC chemokine was isolated, BCA1 (Ref. 17), and later identified as a ligand for the oGPCR BLR1, which has since been renamed CXCR5 (Ref. 18). A fourth, novel class of chemokines called δ-chemokines, or CX_3C chemokines, was discovered by automated high-throughput single-pass sequencing and analysis of a cDNA library constructed from murine choroid plexus[19]. The sequence of one of the cDNA clones exhibited similarity to murine monocyte chemoattractant protein-1 (MCP-1), an α-chemokine. Also, another group independently searched the EST database with known chemokine sequences and identified the same chemokine, which they have termed fractalkine[20]. This ligand was matched to the orphan receptor V28 (renamed CX3CR1)[21]. The ligand for the novel receptor encoded by GPR5 (Ref. 22) has been identified as the single C motif-1 peptide[23] and the receptor renamed as XC chemokine receptor I. The ongoing search for the discovery of novel chemokines will most certainly reveal novel candidates to test with the existing chemokine-like orphan receptors and any additional genes encoding chemokine receptors.

With oGPCR DNAs in hand and with nearly all known ligands assigned, the task now is to use oGPCR DNAs to discover novel ligands[24]. The strategy employed is to express the oGPCR DNA in a cell and apply tissue extracts until a response is observed. The agonist ligand is then purified, synthesized and re-tested. This approach has been most successful in identifying neuropeptides. Peptide ligands often exhibit high-affinity interactions with their receptors, which enables detection at low concentrations and the development of radioligand binding assays. The first success at orphan ligand identification involved a GPCR with sequence identity to the opioid receptors. The natural ligand was identified by two research groups using brain extracts[2,3] and the peptide discovered was 17 amino acids in length, named either orphanin FQ or nociceptin. The peptide contains the tetrapeptide FGGF, which is similar to the motif YGGF of the opioid peptides. Another successful strategy used rat brain fractions that were applied to cells and Ca^{2+} mobilization measured; this succeeded in identifying a novel brain peptide. This peptide and a related peptide (from the same precursor protein) bound to two related oGPCRs and these peptides, which are found in the hypothalamus, function in appetite regulation and satiety control and thus were named orexins[25] (also known as hypocretins[26]). In a similar series of experiments, Hinuma *et al.*[27] measured arachidonate release from CHO cells transfected with the GPR10 (Ref. 28) to identify a novel brain peptide with prolactin-releasing properties at the anterior pituitary. This group has also identified another novel peptide, apelin[29], as the ligand for the receptor APJ (Ref. 30).

The elusive nature of certain labile natural agonists could be a significant hindrance to the discovery of oGPCR ligands, as there is no reason to believe that the remaining oGPCR ligands will all prove to be peptides. An attempt to address this problem involves the use of combinatorial chemistry to generate large libraries of compounds to be tested as surrogate agonists. Although not the physiological solution to the problem, such compounds are tools for probing the pharmacology of an oGPCR. Recently, an interesting variation to this approach was reported. Yeast expressing the human formyl peptide receptor-like oGPCR, FPR2 (Ref. 31), was made dependent on stimulation of this receptor for growth in histidine-free medium and then transfected with a plasmid DNA library designed to express random tridecapeptides. Yeast colonies that were no longer dependent on histidine were judged to have undergone autocrine stimulation and the responsible plasmids recovered. The results yielded a set of six peptides, one of which elicited Ca^{2+} mobilization in HEK293 cells transfected with the FPR2 plasmid.

Ligand-sceening assays

There has been a concerted effort to make ligand identification more efficient by developing cell-based assay systems that have low endogenous GPCR background or report G-protein activation events, or both, in a robust, readily detected manner. The existence of endogenous GPCR signalling systems is important because overexpression of one GPCR can elicit an exaggerated response via other, unrelated and previously unrecognized endogenous GPCRs (Ref. 32), and this could result in false positives. The aforementioned yeast expression system is attractive because of the absence of many endogenous GPCRs. In essence, it involves replacing the endogenous pheromone receptor with a mammalian GPCR and redirecting the pheromone pathway response from a mitogen-activated protein kinase type activation to a biosynthetic circuit, thus allowing the synthesis of histidine. In this case, agonist stimulation allows growth on histidine-free medium. Potential drawbacks of the yeast expression system are the difficulties in expressing some GPCRs achieving effective receptor–G-protein coupling and ligand binding to yeast cell wall components.

Another assay system, which uses mammalian cells, takes advantage of the relatively high expression levels achieved following transfection of oGPCR DNAs so that the endogenous, low-level receptors do not interfere. This system uses the translocation of β-arrestin to receptor sites on the plasma membrane after agonist-mediated receptor activation. Barak *et al.* have shown, using a β-arrestin-2/green fluorescent protein (βarr2–GFP) fusion protein and confocal microscopy, that on agonist stimulation of the β_2-adrenoceptor, βarr2–GFP translocates to the plasma membrane, and that this interaction can be enhanced by co-expression of G-protein-coupled receptor kinase 2 (Ref. 33). This group also showed that similar responses are observed with other receptors coupled to different G proteins, which suggests that the cellular visualization of the agonist-mediated translocation of βarr2–GFP could provide a widely applicable method for detecting the activation of GPCRs.

A system that is useful in measuring GPCR-mediated activation of $G\alpha_q$, $G\alpha_{i/o}$ and $G\alpha_s$ is based on pigment dispersion or aggregation in cultured *Xenopus laevis* melanophores[34,35]. Increases in cAMP ($G\alpha_s$-coupled receptors) or activation of protein kinase C ($G\alpha_q$) lead to pigment dispersion causing darkening of the cells, while decreases in cAMP ($G\alpha_{i/o}$) lead to pigment aggregation near the nucleus and make the cells appear clear[36]. These colour changes are detected readily, however these cells have a substantial complement of endogenous GPCRs, which could confound the results. Overexpression of receptors in melanophores results in changes in the 'basal' signalling and promotes either the clear or the dark cell colour, thus predicting either $G\alpha_{i/o}$ signalling or $G\alpha_q$ or $G\alpha_s$ pathways.

A simpler approach to detecting the activation of multiple types of G proteins uses Gα16 as a universal adapter G protein that can funnel the signal-transduction machinery down a common pathway, such that a single second-messenger response (Ca^{2+} mobilization) can be measured for a given receptor[37]. Heterologous expression of Gα16 allows the coupling of a wide range of GPCRs to phospholipase activity, and thence to Ca^{2+} mobilization. For example, the β_2-adrenoceptor normally couples only to $G\alpha_s$, but when the β_2-adrenoceptor and Gα16 are transiently co-expressed in COS7 cells agonist-dependent stimulation results in inositol phosphate (IP) production[38]. Receptors linked to $G\alpha_s$ (e.g. dopamine D1, vasopressin V_2 and adenosine A_{2A} receptors) or pertussis-toxin-sensitive $G\alpha_i$ (e.g. muscarinic acetylcholine M_2, 5-HT_{1A}, formyl-peptide FPR1 and δ-opioid receptors), when co-transfected with Gα16, also caused concentration-dependent, agonist-mediated IP generation[38]. Other receptors (e.g. thromboxane A_2 and vasopressin V_1) that routinely couple to $G\alpha_q$ and Gα11 to stimulate IP generation were also shown to couple effectively to Gα15 and Gα16 (Ref. 38). However, this coupling is not universal, as the chemokine receptor, CCR1, that effectively couples to $G\alpha_i$ and $G\alpha_q$, failed to couple to Gα16 (Ref. 39).

Other considerations

Recently, new complexities have been added to the general approach to studying orphan GPCRs. For instance, the oGPCR calcitonin receptor-like receptor, has been cloned[40]. The expression of this receptor was consistent with the expression pattern of a calcitonin gene-related peptide (CGRP). The efficient binding of CGRP or amylin, or both, to this receptor required the co-expression of a cofactor protein called receptor activity modifying protein 1 (RAMP1)[41].

Studies have shown that heterodimerization of two GPCR subunits are required for the formation of a functional $GABA_B$ receptor[42–46]. The apparent requirement for two different gene products to create a GPCR signalling entity indicates that the characterization of some oGPCRs might be more complex, perhaps indicating that functional

assays should begin to include co-expression of related oGPCRs.

In principle, the elimination of a GPCR gene from the germline and testing the resulting knockout mice for some change might provide clues to GPCR function, if not ligand identity. For example, when the mouse BLR1 orphan receptor was disrupted, it yielded mice with abnormal primary follicles and germinal centres of the spleen and Peyer's patches, reflecting the inability of B lymphocytes to migrate into B-cell areas[47]. A novel peptide that binds and activates BRL-1 was recently discovered from the EST database[48,49].

In view of the number of novel GPCRs that have been cloned and are continuing to be discovered, it is expected that many endogenous ligands will be discovered. Unquestionably, this will result in an increase in the knowledge of the diversity in intercellular signalling mechanisms and should lead to novel insights into complex or poorly understood human disorders; it will also expand the boundaries of pharmacology. In conclusion, the discovery of the endogenous ligands will help determine the precise physiological role for each oGPCR. As the functions of these novel receptors are uncovered, they could become targets for the development of new pharmacological therapies for diseases not previously considered amenable to pharmacological therapy.

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Anti-GPCR-75Antibodies (GPCR75-100P, GPCR75-101AP and GPCR75-112AP)

Recently a novel human G-protein coupled receptor gene has been characterized and mapped to chromosome 2p16. This gene codes for a 540 amino acid protein in retinal pigment epithelium (RPE) and cells surrounding retinal arterioles. In contrast, the Northern blot data obtained from mouse sections suggest the expression of transcripts in photoreceptor inner segments and I outer plexiform layer. The transcripts of the GPCR-75 gene (7kb) are also found in abundance in brain sections. So far, no mutations in GPCR-75 protein were identified in patients suffering from Doyne's honeycomb retinal dystrophy (DHRD), an inherited retinal degeneration disease that maps to chromosome 2p16 (1).

The GPCR-75 protein is approximately 78 kDa (540 amino acids) protein that is primarily expressed in human retinal pigment epithelium (RPEs). The GPCR-75 sequence analyses suggest the presence of 7 trans-membrane domains, a characteristic feature of GPCR. The protein has putative N-glycosylation sites near the extra cellular N-terminal end of the proteins The protein has a large 3 intra cellular loop which might be the site for interaction of G-proteins. The short carboxy terminal is intracellular and has putative post-translational modification lipid modification sites.

The Anti-GPCR-75-selective antibodies were generated against conserved sequences near N- and C-termini of the protein that are unique to GPCR-75 protein. The polyclonal antibody strongly labels a 78 kDa protein in RPE cell extracts. Anti-GPCR-75-selective antibody is also available in affinity-purified form for confocal, Western blotting and immunocytochemical analyses. *FabGennix Int. Inc.* will also conjugate antibodies with fluorescent probes upon request at extra charge. *FabGennix Int. Inc.* will also provides antibodies against proteins that are involved in retinal degenerative diseases such as various Anti-PDE antibodies, Anti-MERTK, Anti-Phospho-MERTK, EGF-containing fibulin like intracellular protein (EFEMP1), Anti-Myocilin (TIGR), Anti-Bestrophin, Anti-ELVOL4 and a Usher syndrome specific Anti-USH2a antibodies etc. FabGennix Int. Inc employs cyclic peptide methodology for generating antibodies, which results in higher titer and specificity (2). FabGennix Int. Inc., will also provide Western blot positive controls for most of these antibodies in ready-to-use buffer for easy identification of respective proteins. Limited quantities of antigens are also available. Please enquire for their availability before ordering

Catalog #	Host Species	Nature	Cross reactivity	Quantity	volume	Price
GPCR75-100P	Rabbit	Polyclonal antisera	R, M, H	100 ml	100 ul	$ 195.00
GPCR75-101AP	Rabbit	Affinity purified IgG	R, M, H	100 ug	150 ul	$ 225.00
GPCR75-112AP	Rabbit	Affinity purified IgG	R, M, H	100 ug	150 ul	$ 225.00
PC-GPCR75	N/A	WB positive control	Rat	For 5 App	60 ul	$ 75.00
P-GPCR75	N/A	Antigenic peptides	n/a	250 ug	inquire	$ 65.00

R = rat; M = mouse; H = human; C = chicken; monk = monkey ; * not all variants are labeled equally

Immunogen: Synthetic cyclic peptide (GPCR75-101AP = PNATSLHVPHSQEGNSTS-amide; GPCR75-112AP = STSLQEGLQDLIHTATLVTC-amide).

Concentration: GPCR75-101AP; GPCR-112AP IgG concentration 0.75-1.25 mg/ml in 50% antibody stabilization buffer.

Applications: Antibody GPCR75-100/GPCR75-101AP are ideal for WB, IMM and IHC assays. The dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions for specific assay in his/her laboratory. Dilutions: WB > 1:500; Immunoprecipitation & i.p pull-down assays:> 1:250

Reactivity: This antibody detects a single 78 kDa Orphan GPCR75 protein in human RPE cell extracts.

Protocols: Standard protocol for various applications (WB; IMM and IHC) of this antibody is provided with the product specification sheet, however, FabGennix Int. Inc. strongly recommends investigators to optimize conditions for use of this antibody in their laboratories.

78 kDa Orphan Receptor-75 in human RPE cells. Antibody GPCR-100P (1:400)

Form/Storage: The antiserum is supplied in antibody stabilization buffer with 0.02% sodium azide or thimerosal/merthiolate as preservative. The affinity-purified antibodies are purified on antigen-speharose affinity column and supplied as 1-1.25 mg/ml IgG in antibody stabilization buffer containing preservatives with low viscosity and cryogenic properties. For long-term storage of antibodies, store at -20°C. Now these antibodies can be stored at –20°C and used immediately with out thawing. FabGennix Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.45µ filter after every use for long-term storage.

References:

1. Tarttelin E. E., Krischner L. S., Bellingham J., Baffi. J. Taymanas S. E., Gregor E. K., Csaky K., Stratakis C. A., Gregory-Evans C. Y. Biochem. Biophys. Res. Commun. 260, 174-180, 1999.
2. Farooqui, S. M., Brock. W. J., A. Hamdi., Prasad. C. (1991) J. Neurochem. 57, 1363-1369.

* For users who may require large amounts of GPCR75-100P or GPCR75-101AP, please enquire about bulk material discounts.

061901-0020SI 1001Z-rev 10.00

生工有限公司

Rat Taste Receptor 2 (TR2) Antibodies

Rat Taste Receptor 2 (TR2) Antibodies

Cat. # TR21-P, Rat TR2 Control Peptide # 1, SIZE: 100 ug/100 ul
FORM: Œ Soln Œ Lyophilized Lot # 3113P

Cat. # TR21-S, Rabbit Anti-rat TR2 antiserum # 1, SIZE: 100 ul neat antiserum
FORM: Œ Soln Œ Lyophilized. Lot # 38889S

Cat. # TR21-A, Rabbit Anti-rat TR2 Ab # 1 (affinity pure) SIZE: 100 ug
FORM: Œ Soln Œ Lyophilized. Lot # 38889A

Higher vertebrates are believed to possess at least five basic tastes: Sweet, bitter, sour, salty, and unami (the taste of monosodium glutamate). Taste receptor cells that may selectively reside in various parts of the tongue and respond to different tastants and perceive these taste modalities. Circumvallate papillae, found at the very back of the tongue, are particularly sensitive to biter substances. Foliate papillae, found at the posterior lateral edge of the tongue, are sensitive to sour and bitter. Fungiform papillae at the front of the tongue specialize in sweet taste.

Recently, two novel taste receptors, TR1 and TR2, have been cloned with distinct topographical distribution in taste receptor cells and taste buds. TRs are members of a new group of 7 TM domain containing GPCR distantly related to other chemosensory receptors (Ca+-sensing receptor (CaSR, a family of putative hormone receptor (V2R), and metabotropic glutamate receptors). TR1 is expressed in all fungiform taste buds, whereas TR2 localized to the circumvallate taste buds. Both receptors do not co-localize with gustducin.

Source of Antigen and Antibodies

TR1 (rat 840 aa) and TR2 (rat 843 aa) share ~40% homology with each other, and ~30% with CaSR, and 22-30% with V2R pheromone receptors and mGLURs. Rat TR are 7 TM domain containing protein with an extra long N-terminal, extracellular domain (1). A 19 AA Peptide (designated TR21-P; control peptide) sequence near the C-terminus of rat TR2(1) was selected for antibody production. The peptide was coupled to KLH, and antibodies generated in rabbits. Antibody has been affinity purified using control peptide-Sepharose.

Form & Storage

Control peptide Solution is provided in PBS, pH 7.4 at 1 mg/ml (100 ug/100 ul). Antiserum is supplied as neat serum (100 ul soln or lyophilized). Affinity pure antibodies were purified over the peptide-Sepharose column and supplied as 1 mg/ml soln in PBS, pH 7.4 and 0.1% BSA as stabilizer (100 ul in solution or Lyophilized).
The peptides and antibodies also contain 0.1% sodium azide as preservative. Lyophilized products should be reconstituted in 100 ul water and gently mixed for 15 min at room temp. All peptide/antibody

received in solution or

reconstituted from lyophilized vials should be stored frozen at -20oC or below in suitable aliquots. It is not recommended to store diluted solutions. Avoid repeated freeze and thaw.

Recommended Usage

Western Blotting (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure antibody using ECL technique).

ELISA: Control peptide can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

Histochemistry & Immunofluorescence: We recommend the use of affinity purified antibody at 1-20 ug/ml in paraformaldehyde fixed sections of tissues (1).

Specificity & Cross-reactivity

The 19 AA rat TR21-P control peptide is specific for rat TR2. It has no significant sequence homology with TR1 or gustducin or pheromone receptors. Antibody cross-reactivity in various species has not been studied. The TR21-P control peptide is available to confirm specificity of antibodies.

References:

1. Hoon MA et al (1999) Cell 96, 541-555; Lindemann B (1999) Nature Med. 5, 381-382

"**Neat Antisera**" are the unpurified antiserum and it is suitable for ELISA and Western.
"**Affinity pure**" antibodies have been over the antigen-affinity column and recommended for immunohistochemical applications.
"**Control peptides**" can not be used for Western as they are very short peptides. They are intended for ELISA or antibody competition studies.

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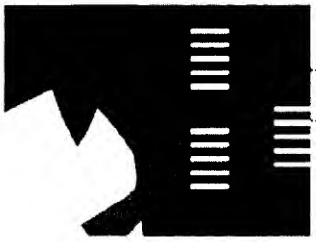

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☐	IOH22493	Human	clone MGC:33224 IMAGE:5267661, mRNA, complete cds.	RDC1
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